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Biotransformation of Acrolein in Rat: Excretion of Mercapturic Acids after Inhalation and Intraperitoneal Injection

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Biotransformation of acrolein (ACR) was studied *in vivo* in the rat following inhalation and ip administration. The major and minor urinary metabolites were 3-hydroxypropylmercapturic acid (HPMA) and 2-carboxyethylmercapturic acid (CEMA), respectively. Male Wistar rats were exposed to ACR, 23, 42, 77 and 126 mg/m^3, for 1 hr. The sum of mercapturic acids HPMA and CEMA excreted within 24 hr after the exposure amounted to 0.87 ± 0.12, 1.34 ± 0.5, 2.81 ± 1.15, and 7.13 ± 1.56 μmol/kg, i.e., 10.9 ± 1.5, 13.3 ± 5.0, 16.7 ± 6.9, and 21.5 ± 4.8% of the estimated absorbed dose, respectively. The dose estimate was based on reported values of minute respiratory volume and respiratory tract retention and was corrected for the ACR-induced changes in minute respiratory volume. In the relevant dose range (8.9 to 35.7 μmol/kg) the portion of mercapturic acids excreted was nearly constant for ip exposed rats. The sum of HPMA and CEMA amounted to 29.1 ± 6.5% of the dose. These results indicate that the deficiency in rat lung metabolism of ACR to acrylic acid previously observed is not compensated by the other detoxication pathway *in vivo*, mercapturic acid formation. The health hazard arising from inhalation of ACR is likely to be higher than that from other routes of exposure.

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Acrolein (ACR) is a toxic chemical formed by combustion and thermal degradation of various organic materials. It is also produced industrially as an intermediate in the manufacture of acrylic acid (AA). The main sources of human exposure to acrolein include tobacco smoke, vehicle exhaust, and the smoke of burning organic materials (EHC, 1992). The molecule of ACR consists of a vinyl group attached to an aldehyde carbonyl. These two groups activate one another for electrophilic reactions. As a consequence, ACR reacts easily with cellular glutathione leading to the excretion of mercapturic acids in urine, namely, *N*-acetyl-*S*-(3-hydroxypropyl)cysteine (3-hydroxypropylmercapturic acid, HPMA) and *N*-acetyl-*S*-(2-carboxyethyl)-cysteine (2-carboxyethylmercapturic acid, CEMA). The former was found in rat urine after subcutaneous application of ACR (Kaye, 1973) whereas the latter was identified in the rat following oral administration (Draminski *et al.*, 1983). Two oxidation products of ACR, AA and glycidaldehyde (GLA), were detected in experiments *in vitro* (Patel *et al.*, 1980). These products undergo further effective metabolic transformation. This may be the reason why they have not been found *in vivo*. Significant differences were found in the hepatic and lung metabolism of ACR. In contrast to rat hepatic preparations, AA was not formed in experiments with microsomes prepared from the rat lung (Patel *et al.*, 1980). The deficiency in this important detoxication pathway in lung may affect significantly the inhalation toxicity of ACR. Although several studies on the inhalation of ACR in rats (Catilina *et al.*, 1966; Potts *et al.*, 1978; Ballantyne *et al.*, 1989; Leach *et al.*, 1987) as well as in other species (Philippin *et al.*, 1970; Lyon *et al.*, 1970; EHC, 1992) have been reported, none of them dealt with biotransformation.

In the light of observed differences between hepatic and lung metabolism of ACR in rat, it seems to be useful to compare biotransformation of ACR following inhalation with that following other routes of exposure. The present study was designed to compare excretion of the primary urinary metabolites of ACR in the rat after inhalation and intraperitoneal injection.

EXPERIMENTAL

Chemicals. Acrolein for synthesis, stabilized with 0.2% hydroquinone, was obtained from Merck Schuchardt (Hohenbrunn bei München, Germany); it was distilled before use. Thiosemicarbazide, AR, ethanol, and ethyl acetate were purchased from Lachema (Brno, Czech Republic). Mercapturic acids, HPMA (Kaye *et al.*, 1972), CEMA (Delbressine *et al.*, 1981), and *N*-Acetyl-*S*-benzylcysteine (benzylmercapturic acid, BZMA) (van Bladeren *et al.*, 1980) were prepared in our laboratory. Their structure and purity (>97%) was confirmed by GLC (as methyl esters), NMR, and mass spectra. Diisopropyl ether (DIPE, 99% pure) was obtained from Aldrich (Steinheim, Germany). Other chemicals were of analytical grade and were used as received.

Intraperitoneal exposure to ACR. Male Wistar rats (Charles River, Germany) weighing 330–420 g were dosed with a single ip injection of 0.47, 0.8, 2.0, and 4.0 mk/kg ACR in saline (2 ml/kg). The control group was nonexposed. Animals were kept individually in glass metabolism cages

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with free access to water and pelleted food (Velaz, Prague, Czech Republic). Five animals were used at each dose level. Urine samples were collected for 24 hr over 2 ml of 0.2 M phosphate buffer, pH 6.5, and stored at −20°C until worked up.

Inhalation of ACR. Male Wistar rats weighing 320–400 g were placed in glass metabolism cages, two animals in each. An atmosphere containing acrolein vapors was prepared by continuous pumping an aqueous solution of acrolein into the stream of 30 liters air/min. The rate of delivery of the ACR solution (1–1.5 μl/sec) was adjusted to obtain desired ACR concentration in air as measured by spectrophotometric method of Manita and Goldberg (1970). Animals were exposed to ACR at 23 ± 3, 48 ± 5, 77 ± 3, or 126 ± 13 mg/m^3 (means ± SD; $n = 3$) for 1 hr. During the exposure animals had free access to water but not to food. After finishing the exposure, animals were supplied with pellets and water *ad libitum* again. Urine was collected, over 2 ml of 0.2 M phosphate buffer, pH 6.5, in three fractions: (i) during exposure, (ii) within 5 hr postexposure, and (iii) from 5 to 23 hr postexposure.

Combined inhalation of ACR and DIPE. The equipment used for combined exposure was the same as that described for inhalation of ACR. Additionally, DIPE was pumped into the stream of air to produce the concentration of 0.9 to 1.3 mg/liter as determined by gas chromatography. After vapor concentrations of both ACR and DIPE became stabilized, six rats were placed into the metabolism cages, two rats in each. Immediately after terminating the 1-hr exposure, blood samples (ca. 100 μl) were taken from the ophthalmic plexus and analyzed for DIPE as described below.

Determination of ACR in air. Air samples (1 liter) were bubbled through a 0.2% ethanolic solutions of thiosemicarbazide at a flow rate of 120 to 170 ml/min. Absorbance at 290 nm was measured after standing for at least 20 min (Manita and Goldberg, 1970).

Determination of DIPE in air. Air was sampled into 0.5-liter bottles. Aliquots (1 ml) were taken through a silicone rubber septum using a gas-tight syringe and analyzed by gas–liquid chromatography with a flame ionization detector, on a glass column 1.5 m × 3 mm i.d. packed with 0.8% Carbowax 20 M on Carbopack BH, 80/100 mesh (Supelco). The flow rates of nitrogen carrier gas and hydrogen were 30 and 38 ml/min, respectively. Column, injector, and detector temperatures were 148, 180, and 160°C, respectively. Calibration mixtures were prepared by injecting 5 to 10 μl of DIPE into a 0.5-liter dry bottle through a septum. Before analysis, calibration mixtures were diluted with air in the gas-tight syringe 2 to 10 times.

Determination of DIPE in blood. Blood samples were taken from the ophthalmic plexus using 100 μl glass capillary and transferred into preweighed screw-capped vials. The vials were sealed with silicone rubber septa covered with a Teflon foil, weighed, and kept at 40°C for at least 20 min. Aliquots (1 ml) of the head space were analyzed by gas chromatography under the conditions described for airborne DIPE.

Determination of HPMA and CEMA in urine. Urine samples (4.75 ml) were diluted with 8 ml of deproteinization reagent (Seutter-Berlage *et al.*, 1977), 0.25 ml of BZMA solution (1.27 mg/ml in 1:3 ethanol:water) was added, and the solution was saturated with ammonium sulfate, acidified to pH 2 by 4 M hydrochloric acid, and extracted twice with 10 ml of ethyl acetate. Combined ethyl acetate extracts were reacted with slight excess of ether solution of diazomethane, dried with anhydrous magnesium sulfate, filtered, and evaporated to dryness *in vacuo*. The residues were dissolved in 1.5 ml ethyl acetate, 0.5 ml acetanhydride, and 20 μl pyridine. The solutions were heated on a 60°C water bath for 40 min, cooled in water, and allowed to stand for 2 hr at room temperature. Additional 2 ml of ethyl acetate was added along with 2 ml of water and ca. 0.2 g of sodium bicarbonate. The mixture was vortexed vigorously several times during 30 min to hydrolyze excess acetanhydride. The organic layer was washed with 1.5 ml of 0.1 M HCl and 1.5 ml of water and dried with magnesium sulfate. Finally, the samples were filtered and concentrated to volume of 0.5 to 1 ml before analysis. Aliquots (2 μl) were analyzed by gas chromatography on a fused silica capillary column Spira KI 19 (25 m × 0.2 mm; semipolar silicone-bonded phase; Lachema Brno, Czech Republic) with a flame ionization detector. The flow rate of helium carrier and makeup gas was 0.35 and 35 ml/min, respectively. Hydrogen flow rate was 38 ml/min. The injector and detector temperatures were 260 and 160°C, respectively. Column temperature was held at 202°C for 32 min and thereafter programmed to 250°C at 3°C/min. The stream splitter was adjusted to 1:30. Calibration curves were linear from 25 to 500 μmol/liter for both HPMA and CEMA.

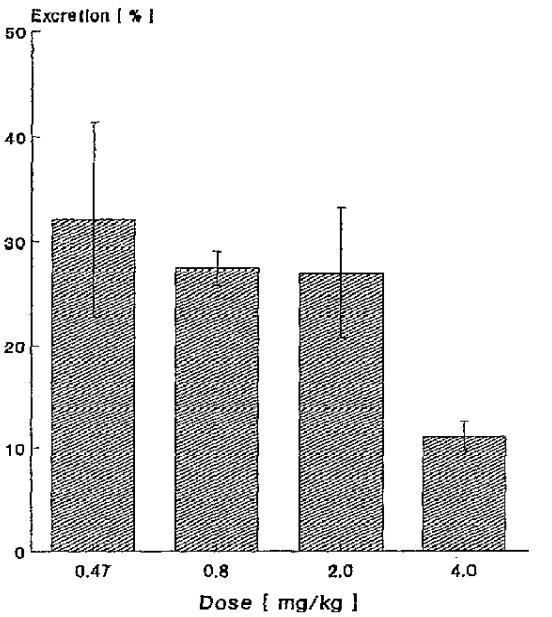

FIG. 1. Metabolic conversion of ACR to mercapturic acids, HPMA + CEMA after a single intraperitoneal injection. Means ± SD; $n = 5$, except for the dose of 4 mg/kg when $n = 3$.

RESULTS

Two mercapturic acids, HPMA and CEMA, were identified in the urine of rats exposed to ACR either by inhalation or ip injection. In both cases, HPMA was more abundant. The structure of these metabolites was confirmed by GC/MS comparing mass spectra of their derivatives with those of authentic standards. The spectrum of CEMA dimethyl ester has been reported previously (Delbressine *et al.*, 1981); mass spectrum of AHPC O-acetylated methyl ester showed a molecular ion at *m/z* 277 and fragment peaks (*m/z* to relative intensity) at 218/57 $(M - CH_3CONH_2)^+$; 186/15 $(M - CH_3CONH_2 - CH_3OH)^+$; 176/10 $(M - COOCH_3 - CH_2CO)^+$; 158/23 $(M - CH_3CONH_2 - CH_3COOH)^+$; 43/100 $(CH_3CO)^+$.

In rats given ip ACR, 0.47 to 2.0 mg/kg (8.9–35.7 μmol/kg), the sum of HPMA and CEMA excreted for 24 hr accounted for 29.2 ± 6.5% of the dose. At the highest dose applied (4 mg/kg, 71.4 μmol/kg), the metabolic conversion to mercapturic acids decreased to 11% (Fig. 1); two of five rats were anuretic. CEMA amounted less than 10% of the

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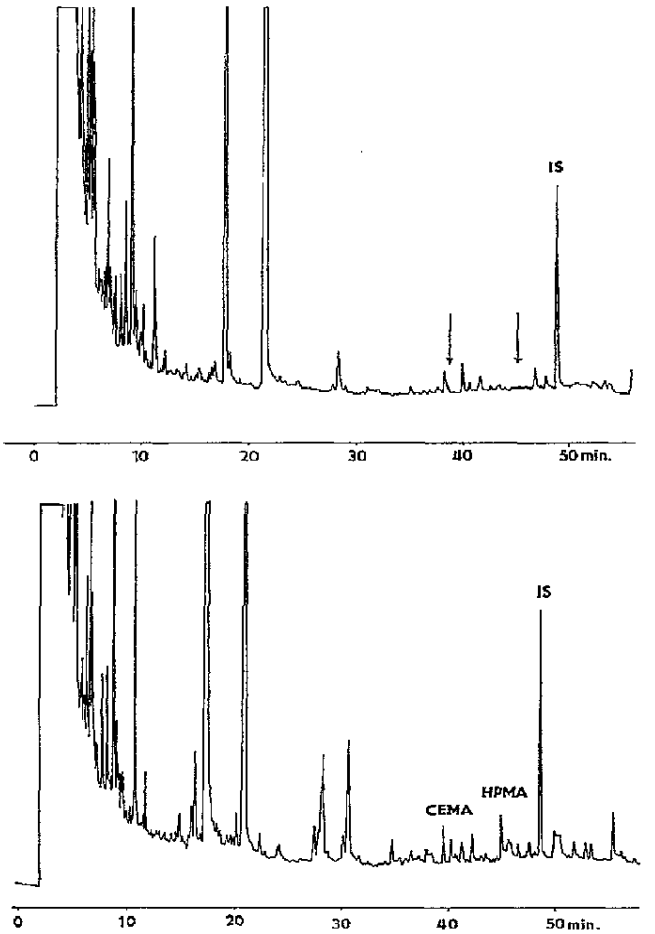

FIG. 2. Typical chromatogram of rat urine from control (top trace) and ACR exposed rats (bottom trace).

mercapturic acids excreted. In many urine samples, particularly from rats exposed to low doses of ACR, CEMA was not quantifiable by the method used. In these cases, the amount of HPMA was taken as total of mercapturic acids.

In the inhalation experiments, the metabolic pattern of ACR was very similar to that in ip exposed rats with CEMA being a minor urinary metabolite amounting to not more than 10% of the two mercapturic acids detected. Typical chromatograms of urine from the exposed and control rats are shown on the Fig. 2. Excretion of mercapturic acids, HPMA + CEMA within 24 hr after the exposure to various concentrations of inhaled ACR is shown on the Fig. 3. Of the three collected fractions of urine, the main portion of the metabolites was found in fraction ii collected during the first 5 hr postexposure. Fractions i collected during the exposure contained mostly saliva due to extensive salivation of the exposed animals (creatinine content in this fraction was less than 0.23 mg/ml). Fraction iii did not contain detectable amounts of HPMA and CEMA except few cases when very little or no urine was collected during first 5 hr postexposure.

To evaluate the metabolic conversion of inhaled ACR to HPMA and CEMA, the absorbed dose had to be estimated. The dose was then calculated according to the equation

$$D = R \cdot c_{ACR} \cdot V \cdot t_e/m,$$

where

D = absorbed dose per rat (μmol)
R = retention in the respiratory tract (0.83; Egle, 1972)
c_{ACR} = concentration of ACR (μmol/liter)
V = minute respiratory volume (0.1 liter per rat min^{-1}; Spector, 1956)
t_e = duration of exposure (min).

The uptake calculated was corrected for possible variations in lung ventilation due to exposure to the strongly irritating ACR vapors (Babiuk *et al.*, 1985). To evaluate the influence of ACR on minute respiratory volume, rats were exposed concomitantly to DIPE as a marker of pulmonary uptake. The blood levels of DIPE at the end of 1-hr exposure reflect the variations in the uptake resulting from the differences in ventilation. However, the effect of coinhaled ACR on blood levels of DIPE was relatively small. Surprisingly, the only significant change in blood level (increase) was found at ACR concentration of 23 mg/m^3, whereas at higher

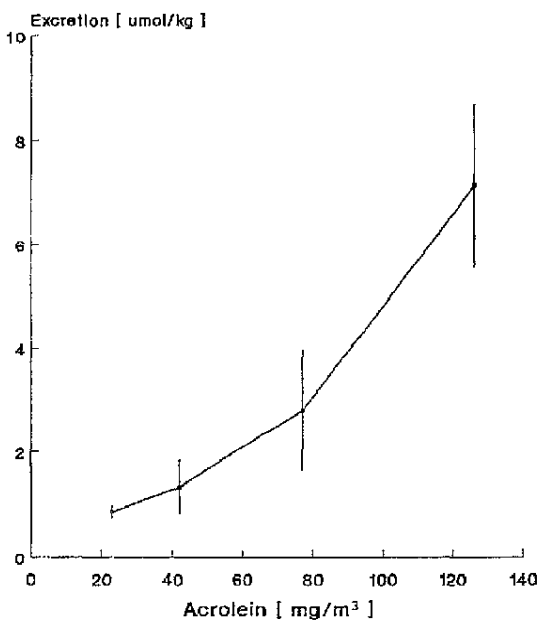

FIG. 3. Excretion of mercapturic acids, HPMA + CEMA, for 24 hr after a 1-hr inhalation of ACR. Means ± SD; $n = 6$.

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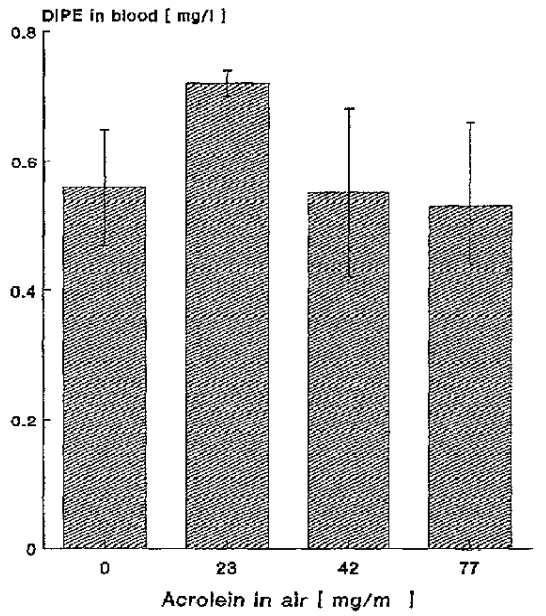

FIG. 4. Blood levels of DIPE at the end of 1-hr-long inhalation of DIPE (1 mg/liter) depending on the concentration of coinhaled ACR. Means ± SD; $n = 6$.

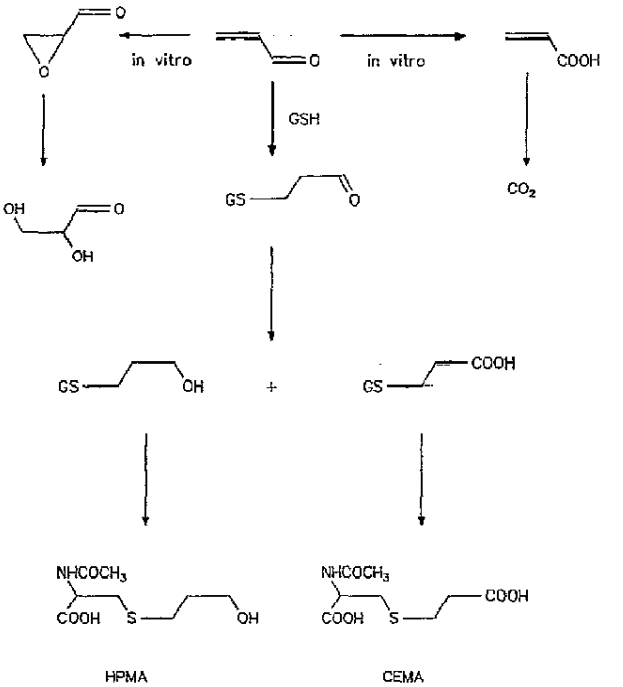

FIG. 6. Metabolic pathways of acrolein.

concentrations no significant effect was observed (Fig. 4). Actual values of inhaled DIPE concentration varied from 0.9 to 1.3 mg/liter in various experiments, but were stable during each particular experiment. The blood levels of DIPE were therefore recalculated to an arbitrary concentration in inhaled air, 1 mg/liter.

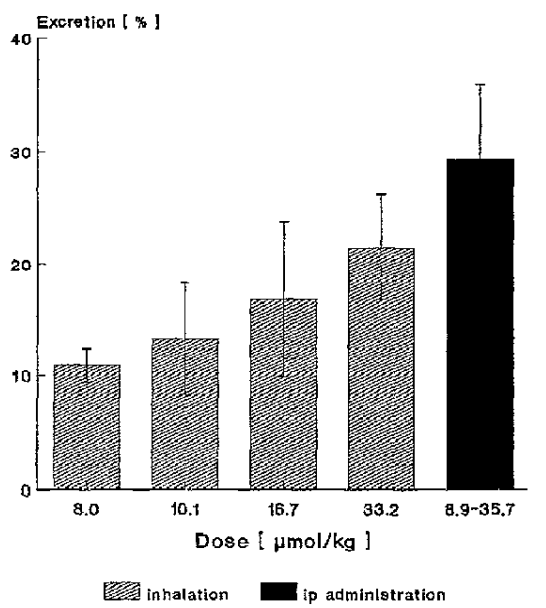

FIG. 5. Excretion of mercapturic acids, HPMA + CEMA, after inhalation and ip exposure to ACR. Percentage of the dose. Means ± SD; $n = 6$ and 15 for inhalation and ip administration, respectively.

The metabolic yields of HPMA and CEMA from inhaled ACR are shown on the Fig. 5. They tend to be somewhat lower than that of ACR administered intraperitoneally. The difference was statistically significant at low ACR concentrations (23 and 48 mg/kg).

DISCUSSION

Conjugation with glutathione is one of two major detoxication pathways of ACR (Fig. 6). The other one, oxidation to AA, occurs mainly in liver but does not occur in rat lungs as determined by experiments *in vitro* (Patel *et al.*, 1980). The extent of ACR oxidation to AA is not directly measurable *in vivo* due to the rapid metabolic degradation of AA (de Bethizy *et al.*, 1987; Winter *et al.*, 1992). Nevertheless, differences in the metabolism of ACR depending on the routes of exposure can be followed by measuring the excretion of mercapturic acids resulting from the conjugation with glutathione. Mercapturic acid excretion expressed as percentage of the estimated dose of inhaled ACR tended to increase with the inhaled concentration and was slightly but significantly lower than that of intraperitoneally exposed rats, particularly, at lower concentrations. Excretion of HPMA + CEMA after an ip administration (percentage of dose) was stable in the dose range of 0.47–2.0

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mg/kg but decreased significantly at the highest dose of 4 mg/kg (Fig. 1).

At high ip doses focal simple hyperplasia of the urinary bladder was reported (Sakata *et al.*, 1989). Also, acute cytotoxicity for the rat urinary bladder mucosa was found when ACR was instilled directly into the bladder lumen (Chaviano *et al.*, 1985). It is therefore not surprising that the excretion of urine may be impaired after high intraperitoneal doses. Thus, decreased excretion of mercapturic acids at the dose of 4 mg/kg may be explained by an impaired excretion of urine rather than by capacity limitation of glutathione conjugation.

Breathing acrolein, like other irritating compounds, causes a significant decrease in the respiratory rate (Babiuk *et al.*, 1985) during inhalation. A marked decrease in the respiratory rate appeared to be evident on observation. At the same time, a marked increase of tidal volume was observed. To quantify the influence of ACR on lung ventilation we designed an experiment using DIPE as a marker of the pulmonary uptake. Since DIPE is a poorly metabolized compound, it was expected that blood level of DIPE at the end of 1-hr inhalation depended solely on the pulmonary uptake. In fact, blood levels found for DIPE show relatively low variability; therefore, they seem to be a good indicator of changes in the uptake, although they do not reflect an absolute value. It is surprising that the decrease in respiratory rate is fully compensated by an increased tidal volume even at 77 mg/m^3 ACR. Moreover, at the lowest ACR dose, 23 mg/m^3, blood level of DIPE was significantly higher than that of control indicating an increase in minute respiratory volume. Therefore, the dose estimate was corrected accordingly. It remains to be established whether the full compensation of a decrease in the respiratory rate caused by exposure to irritating substances by increased tidal volume is a general phenomenon or is specific for ACR. We believe that the approach used here, i.e., determination of blood levels of an indicator of pulmonary uptake, may be useful in this type of studies.

It does not appear that the deficiency in metabolic oxidation of ACR to AA in the rat lung is compensated by the other major detoxication pathway. It is therefore likely that inhaled ACR is increasingly metabolically activated to glycidaldehyde and/or covalently bound to proteins (Marrinello *et al.*, 1984; Beauchamp *et al.*, 1985). In fact, inhalation LD50 values estimated from reported LC50's for rat, lung ventilation, and retantion of ACR in the respiratory tract are much lower than LD50's reported for other routes of exposure (Table 1). Although there may be other reasons why the toxicity arising from inhalation of ACR is higher than that from other routes of exposure, the lack of AA formation from ACR in rat lung as well as lower metabolic conversion to HPMA and CEMA after inhalation compared to ip administration seems to add significantly to the inhalation toxicity of ACR.

TABLE 1
Acute Toxicity of ACR in Male Rat after Inhalation, Intraperitoneal, Oral Administration

Exposure	LC50 (mg/m^3)	LD50 (mg/kg)
Inhalation, 1 hr	65	0.95[a]
Inhalation, 30 min	95–217	0.67–1.5[b]
Inhalation, 10 min	750	1.8[c]
Oral		46[d]
Intraperitoneal		~10[e]

Note. Inhalation LD50's are estimates based on experimental LC50's, lung ventilation, and retention in the respiratory tract.

[a] Ballantyne *et al.*, 1989.
[b] Potts *et al.*, 1978.
[c] Catilina *et al.*, 1966.
[d] Smyth *et al.*, 1951.
[e] Draminski *et al.*, 1983.

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